

STUDIES ON ERGOT INFECTION IN GRAMINEOUS HOSTS<sup>1</sup>W. P. CAMPBELL<sup>2</sup>

## Abstract

Four hundred and twenty-one isolates of *Claviceps purpurea* from 38 different host species were cultured on an artificial medium. The conidial cultures were used to inoculate rye, wheat, and barley growing in the greenhouse. All cultures, except one from *Glyceria borealis*, infected the three host species. Honeydew from rye was used to inoculate 46 gramineous species in the field and in the greenhouse. Every one of these grasses became infected in both locations. It is shown that all indigenous and forage grasses constitute a reservoir of ergot inoculum for rye, wheat, and barley, and that these cereals can be infected provided the environmental conditions are such that inoculum will be disseminated at the time that the cereal crops are in blossom.

## Introduction

*Claviceps purpurea* (Fr.) Tul. is known to parasitize many species and genera of the Gramineae, including our common forage and cereal crops. This fungus attacks the inflorescence at flowering time and some of the grain in the head is replaced by sclerotia of the parasite. Data from extensive field surveys in Alberta showed that ergot was prevalent on wheat, barley, and rye in 1953, but in 1954 it was comparatively scarce on these cereals. In 1953 ergot was found in varying amounts in 13.3% of 645 fields of wheat, 7.2% of 376 fields of barley, and 53.4% of 58 fields of rye. In 1954 ergot was recorded in 1.6% of 626 fields of wheat, 4% of 351 fields of barley, and 15.4% of 39 fields of rye. The parasite was common on the roadside grasses in both years. The inoculum was thus present each year but caused more infection of cereals during the former season than in the latter. The official yearly inspection of carload lots of rye (3) serves to emphasize the sharp variability in the seasonal incidence of ergot. In the 1941-42 crop year 0.9% of the carload lots were graded ergoty, while in 1942-43 15.3% were so graded. For the 1950-51 crop year 7.7% of the carload lots graded ergoty, whereas in 1951-52 only 0.2% were ergoty. In 1952-53 the incidence was 10.1%.

The weather at flowering time has been shown to be an important factor in influencing infection by ergot (10) and this has probably been one of the causes of the wide variability in the incidence of ergot in cereals. However, other factors may also be involved and one of these is the host range of the pathogen.

There are conflicting reports in the literature concerning host specificity and the ability of the fungus to pass from one host species to another. Stäger (5, 6, 7, 8, 9) tested a large number of host species with several isolates of the fungus. He concluded that not only were there several species of *Claviceps* but that within *C. purpurea* itself there were several races, each

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Contribution No. 1590 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

<sup>2</sup>Plant Pathology Laboratory, Edmonton, Alberta.

with its own distinct host range. Race I (from rye) attacked barley, wheat, *Anthoxanthum odoratum*, *Hierochloë borealis*, *Arrhenatherum elatius*, *Dactylis glomerata*, *Hordeum murinum*, *Festuca pratensis*, *Phalaris arundinacea*, *Holcus mollis*, *Brizia media*, *Calamagrostis arundinacea*, *Bromus sterilis*, *Poa caesia*, *P. pratensis*, *P. sudetica*, *P. hybrida*, *P. compressa*, *P. alpina*, and *P. concinna*. On the last two of these hosts there was only a transitory infection which did not produce sclerotia. Race II (from *Brachypodium sylvaticum*) attacked only *Poa annua* and *Milium effusum*. Race III (from *Lolium perenne*) attacked only *Bromus erectus*, *Lolium italicum*, *L. temulentum*, and *L. rigidum*. Békésy (1), however, found that *Bromus erectus*, *Lolium perenne*, and *Melica uniflora* were all susceptible to ergot from rye, although Stäger had listed them as immune. Békésy also successfully infected rye with ergot from *Lolium perenne*. Brown (2) has shown that a single strain of *C. purpurea* could attack rye, wheat, barley, oats, *Bromus inermis*, *Agropyron* spp., *Elymus* spp., and *Poa pratensis*. Brown (2) and Fyles (4) have demonstrated that *Claviceps* from *Zizania aquatica* will not infect cereals or other grasses.

Stäger (5) used three methods of inoculation: spraying the heads with a spore suspension, dipping the heads into a spore suspension, and dropping the spore suspension into the florets while the glumes were held apart. Many of the host species were inoculated by only one method and when negative results were obtained no second attempt was made to infect the plant in question (5). *Triticum spelta* (5), for example, was inoculated once with a single strain of *C. purpurea* and when it did not become infected it was considered to be immune.

Ergot sclerotia are usually prevalent in the grasses by the roadside and in the headlands of cereal fields. It was of interest to determine if ergot cultures from these grasses were able to infect the cereal species.

### Materials and Methods

During the autumns of 1953, 1954, and 1955, 421 samples of ergot were collected from wild and cultivated hosts. These samples were obtained at different points in Canada, chiefly in the Prairie Provinces. Some collections from wild grasses came from areas remote from any cultivation. The hosts from which the collections were taken and their geographic distribution are shown in Table I.

The sclerotia were surface sterilized by shaking for 30 seconds in 0.1% aqueous mercuric chloride, rinsed in sterile water, and cut into small pieces with a sterile knife. The pieces were plated out on an acidified medium of the following formulation: 10 g. dextrose, 5 g. malt extract, 5 g. yeast extract, 20 g. agar, and 1 liter of water. When the mycelium grew out of the ergot fragments it was transferred to slants of potato-dextrose agar on which satisfactory sporulation occurred. Spore suspensions from these tubes were used as inoculum for the test host plants.

In attempts to secure satisfactory infection, four methods of inoculation were tested on Thatcher wheat, Newal barley, and Prolific rye. These

methods were: heads of host dipped into a spore suspension; spore suspension injected into floral cavity; inoculum sprayed on the heads; and the tips of glumes clipped away before heads sprayed with inoculum. The method finally adopted varied according to the host plant. For rye and wheat the tips of the glumes were removed when the anthers appeared, and the heads were then sprayed by atomizer with a suspension of the conidia. To secure infection in barley it was necessary to inoculate the heads as they were emerging from the leaf sheath. No infection resulted if inoculation was delayed until the anthers appeared. The sheath was cut back, the awns and glume tips clipped off, and the spore suspension was sprayed on the heads. It was occasionally necessary with all these hosts to repeat the inoculation to obtain infection. All inoculations were done with an atomizer which was thoroughly washed with 0.1% aqueous solution of mercuric chloride and rinsed with water before spores of another isolate were passed through it. Two sets of controls were used. In one set an isolate of *C. purpurea* that produced only white sclerotia was used as inoculum and in the second set the heads were sprayed with sterile water. Humidity chambers were not used since they were found to be unnecessary. The greenhouse was fumigated regularly to rid it of insects.

In a field test the following grasses were inoculated with conidial suspensions of ergot from rye: *Agropyron cristatum* (L.) Gaertn., *A. desertorum* (Fisch.) Schult., *A. elongatum* Host., *A. inerme* (Scribn. & Smith) Rydb., *A. intermedium* (Host) Beauv., *A. riparium* Scribn. & Smith, *A. trachycaulum* (Link) Malte, *A. triticeum* Gaertn., *Agrostis alba* L., *A. tenuis* Sibth., *A. palustris* Huds., *Alopecurus occidentalis* Scribn. & Tweedy, *Bromus erectus* Huds., *B. inermis* Leyss., *B. latiglumis* (Shear) Hitchc., *Calamagrostis canadensis* (Michx.) Beauv., *Dactylis glomerata* L., *Danthonia intermedia* Vasey, *Elymus canadensis* L., *E. interruptus* Buckl., *E. junceus* Fisch., *E. sibericus* L., *E. virginicus* L., *Eragrostis minor* Host., *E. pilosa* (L.) Beauv., *Festuca elatior* var. *arundinacea* (Schreb.) Wimm., *F. rubra* L., *F. rubra* var. *commutata* Gaud., *F. scabrella* Torr. var. *major* Vasey, *Koeleria cristata* (L.) Pers., *Phalaris arundinacea* L., *Phleum pratense* L., *Poa ampla* Merr., *P. canbyi* (Scribn.) Piper, *P. compressa* L., *P. cusickii* Vasey, *P. interior* Rydb., *P. pratensis* L., *P. pratensis* 'Merion', *Polypogon monspeliensis* (L.) Desf., *Schedonnardus paniculatus* (Nutt.) Trel., *Setaria glauca* (L.) Beauv., *Sitanion hystrix* (Nutt.) J. G. Smith, *Sphenopholis obtusata* (Michx.) Scribn., *Sporobolus neglectus* Nash, *Stipa spartea* Trin., and *Stipa viridula* Trin. The same group of grasses was also inoculated under greenhouse conditions.

### Experimental Results and Discussion

Ergot samples were collected from a wide range of hosts. The number of collections on each host and their regional distribution are shown in Table I. All but one of these isolates produced infection on rye, wheat, and barley; the lone exception was one of the three from *Glyceria borealis*, which did not infect any of the test species. The other two collections from this host did

TABLE I  
GEOGRAPHIC DISTRIBUTION OF THE HOSTS FROM WHICH  
*Claviceps purpurea* WAS COLLECTED

| Host                                            | Creston,<br>B.C. | Peace River<br>district,<br>Alberta,<br>and<br>British<br>Columbia | Central<br>Alberta | Southern<br>Alberta | Saskat-<br>chewan | Mani-<br>toba | Southern<br>Ontario |
|-------------------------------------------------|------------------|--------------------------------------------------------------------|--------------------|---------------------|-------------------|---------------|---------------------|
| <i>Agropyron albicans</i> Scribn. & Smith       |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Agropyron cristatum</i> (L.) Gaertn.         |                  |                                                                    | 3                  |                     |                   |               |                     |
| <i>Agropyron dasystachyum</i> (Hook.) Scribn.   |                  | 6                                                                  | 2                  |                     |                   |               |                     |
| <i>Agropyron inerme</i> (Scribn. & Smith) Rydb. |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Agropyron repens</i> (L.) Beauv.             |                  | 3                                                                  | 28                 |                     | 1                 | 1             | 1                   |
| <i>Agropyron smithii</i> Rydb.                  |                  | 4                                                                  | 1                  |                     |                   |               |                     |
| <i>Agropyron subsecundum</i> (Link) Hitchc.     |                  | 8                                                                  | 4                  |                     |                   |               |                     |
| <i>Agropyron trachycaulum</i> (Link) Malte.     |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Agropyron</i> sp.                            |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Agrostis alba</i> L.                         |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Agrostis exarata</i> Trin.                   |                  | 1                                                                  |                    |                     |                   |               |                     |
| <i>Arrhenatherum elatius</i> (L.) Presl.        |                  | 1                                                                  |                    |                     |                   |               |                     |
| <i>Avena fatua</i> L.                           |                  |                                                                    | 2                  |                     |                   |               |                     |
| <i>Avena sativa</i> L.                          |                  |                                                                    | 2                  |                     |                   |               |                     |
| <i>Bromus ciliatus</i> L.                       |                  | 2                                                                  | 1                  |                     |                   |               |                     |
| <i>Bromus inermis</i> Leyss.                    |                  | 27                                                                 | 33                 |                     | 3                 | 3             |                     |
| <i>Bromus punpelliianus</i> Scribn.             |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Calamagrostis canadensis</i> (Michx.) Beauv. |                  | 11                                                                 | 11                 |                     |                   |               |                     |
| <i>Calamagrostis inexpansa</i> A. Gray          |                  |                                                                    | 2                  |                     | 1                 |               |                     |
| <i>Calamagrostis neglecta</i> (Ehrh.) Gaertn.   |                  |                                                                    |                    |                     | 1                 |               |                     |
| Mey. & Schreb.                                  |                  |                                                                    |                    |                     |                   |               |                     |
| <i>Calamovilfa longifolia</i> (Hook.) Scribn.   |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Dactylis glomerata</i> L.                    |                  |                                                                    |                    |                     |                   |               | 1                   |
| <i>Elymus canadensis</i> L.                     |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Elymus glaucus</i> Buckl.                    |                  |                                                                    | 2                  |                     |                   |               |                     |
| <i>Elymus innovatus</i> Beal.                   |                  | 26                                                                 | 14                 |                     |                   |               |                     |
| <i>Festuca rubra</i> L.                         |                  | 1                                                                  |                    |                     |                   |               |                     |
| <i>Glyceria borealis</i> (Nash) Batchelder      |                  |                                                                    | 3                  |                     |                   |               |                     |
| <i>Hordeum vulgare</i> L.                       |                  | 2                                                                  | 23                 | 1                   | 8                 | 1             |                     |
| <i>Lolium perenne</i> L.                        |                  |                                                                    |                    |                     |                   |               | 1                   |
| <i>Phleum pratense</i> L.                       |                  | 4                                                                  | 9                  |                     |                   |               | 1                   |
| <i>Poa pratensis</i> L.                         |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Secale cereale</i> L.*                       | 1                | 11                                                                 | 29                 | 6                   | 5                 | 4             | 1                   |
| <i>Spartina gracilis</i> Trin.                  |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Stipa spartea</i> Trin.                      |                  |                                                                    | 1                  |                     |                   |               |                     |
| <i>Stipa viridula</i> Trin.                     |                  |                                                                    | 2                  |                     |                   |               |                     |
| <i>Triticum aestivum</i> L.                     | 1                |                                                                    | 80                 |                     | 6                 | 2             |                     |
| <i>Triticum durum</i> Desf.                     |                  |                                                                    |                    |                     | 1                 |               |                     |
| <i>Triticum monococcum</i> L.                   |                  |                                                                    | 1                  |                     |                   |               |                     |
| Total                                           | 2                | 107                                                                | 263                | 7                   | 26                | 11            | 5                   |

\*Includes two collections of albino sclerotia and one collection from tetraploid rye.

infect rye, wheat, and barley. The check plants inoculated with the albino strain produced only white sclerotia and the series sprayed with sterile water remained healthy. The average time between inoculation and the first appearance of honeydew was 6.9 days for barley, 8.5 days for rye, and 10.3 days for wheat.

The grass species inoculated with ergot from rye all became infected, both in the field and in the greenhouse.

These results are at variance with those secured by Stäger, who reported failure to secure infection in many instances of cross inoculation between species of the Gramineae. On the basis of these negative results Stäger has postulated the existence of various physiologic races of *C. purpurea* which are host specific. In the present study variability in the conidial colonies was noted, which indicates that physiologic races, in the cultural sense of the term, do indeed exist. However, no evidence was found that these races were species specific.

It is possible that in many cases Stäger might have secured infection if he had used more adequate samples or repeated his inoculations. In the present work, plants were inoculated and if no infection occurred the inoculations were repeated. In the case of the noninfective isolate from *G. borealis* inoculation was repeated about 15 times with fresh host specimens for each attempt. Stäger often based his conclusions on the negative results from a single test, e.g. *Nardus stricta*, *Triticum spelta*, *Lolium italicum*, *Glyceria distans*, and *Molinia coerulea* (5).

Moreover, the present work has indicated that the techniques of inoculation are of importance. Stäger (5) used three methods: spraying the heads with an inoculum of spores suspended in water, prying the glumes apart and spraying the inoculum into the florets, and dipping the heads into the inoculum. The author found in preliminary work that dipping the heads into the inoculum was useful in securing infection with only a few species, e.g. *Bromus inermis* and rye. Those hosts with stiff glumes or ones in which the glumes were hairy or waxy were not readily infected by this method of inoculation. Any hosts in which the glumes did not open wide tended to trap air in the florets and exclude the spore suspension. In many cases spraying the heads with a spore suspension was also ineffective because the florets were closed and the spores were excluded. It was often found necessary to inject the inoculum into the florets with a hypodermic syringe or clip the tips of the glumes so that the spores could be blown in with an atomizer. Since Stäger did not use all three of his own methods in attempts to get infection it is not surprising that he frequently failed to secure it.

It is interesting to note that, in this work, ergot from *Lolium perenne* infected rye. This is not in agreement with Stäger's results (5), but does support the findings of Békésy (1), who infected rye with ergot from *L. perenne* and *L. perenne* with ergot from rye.

The morphology and developmental rhythm of the host are probably of the utmost importance in determining whether there will be any infection in nature. It is to be expected that a host in which the glumes do not open at all, or one in which they are open for a very short period of time, would provide only slight opportunity for infection. Also, a particular variety in which fertilization takes place while the head is still enclosed by the leaf sheath may be too far advanced for infection by the time the florets are exposed. On the other hand, an open pollinated plant in which the glumes are open for an extended period of time might offer the greatest opportunity for infection (9).

No evidence could be found that the percentage infection in roadside and headland grasses fluctuated appreciably from year to year as was noted in the cereal crops. Hence it appears that, although inoculum is potentially available each year, environmental conditions do not always favor effective dissemination from the grasses to the grain fields. However, it is apparent that the weed and roadside grasses constitute important reservoirs of ergot inoculum for the infection of our cereal crops. If the disease is to be controlled in the field sclerotia must be prevented from maturing in these grasses.

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